23. *Difference of Chromosome Exchanges Due to Mitomycin C between Normal and Pericentric Inv(9) Cells*

By Yukimasa SHIRAISHI

Department of Anatomy, School of Medicine, Kanazawa University,
Kanazawa 920, Japan

(Communicated by Sajiyo MAKINO, M. J. A., June 14, 1977)

Several reports are available for the finding that mitomycin C (MMC) induces non-random chromosome exchanges in human cells (Nowell 1964; Cohen and Shaw 1964; Brøgger and Johansen 1972). It has been shown that the majority of exchanges occurs preferentially between the constitutive heterochromatin of homologous chromosomes, especially in the secondary constriction regions of chromosomes 1, 9 and 16. This may be a result of the association of these chromosome regions during interphase, either through regular somatic pairing (Rao and Natarajan 1967) or through a particular gathering effect of MMC (Kobayashi 1960). There is little information on the mitotic feature of the somatic pairing in chromosomes with rearrangements, particularly with pericentric inversions of mammalian cells. The present study was attempted to test the effect of MMC on cells with pericentric inv (9), one of the polymorphic variations in man (Hansmann 1976; Shiraishi and Makino 1977), and to compare the frequency of MMC-induced exchanges between normal and pericentric inv (9) cells.

Materials and methods. Heparinized blood samples from a normal person and a patient with pericentric inv (9) were cultured for 72 hrs in PHA-containing RPMI 1640 medium supplemented with 15% fetal calf serum at 37°C. The cultures were treated with mitomycin C (MMC) at concentrations (1×10^{-7} g/ml and 5×10^{-7} g/ml) for the last 24 hrs before the fixation. Colcemide ($0.02 \mu\text{g/ml}$) was added to the cultures 90 min prior to fixation. Chromosome preparations were processed according to the conventional air-drying method. The pericentric inv (9) patient, phenotypically normal but having experienced several abortions, was previously described (Shiraishi and Makino 1977).

Results and discussion. The chromosome aberrations observed in this study were of exchange- and break-types. The chromosomes involved in the exchanges were found in most cases associated at the centromere regions. The breaks observed included chromatid- as well

Table I. Chromosome aberrations after treatment with mitomycin C(MMC)

Chemicals (g/ml)	Subjects	No. of cells observed	Chromosome aberrations		Cells with chromosome aberrations (%)
			Exch*	Breaks**	
0	normal	200	0	13	4
MMC 1×10^{-7}	normal	200	36	81	52
MMC 5×10^{-7}	normal	200	102	107	76
0	inv (9)	200	0	27	9
MMC 1×10^{-7}	inv (9)	200	24	101	61
MMC 5×10^{-7}	inv (9)	200	71	142	84

* Exch: Chromatid exchanges.

Breaks: Breaks including chromatid and chromosome breaks.

Table II. Number of chromatid exchanges and breaks on individual chromosomes exposed to 5×10^{-7} g/ml MMC for the last 24 hrs in cultures

Chromosome number or groups	Normal		inv (9)	
	No. of Exch	No. of Breaks	No. of Exch	No. of Breaks
1	20	12	16	13
2	6	14	5	19
3	3	9	3	7
B	3	11	2	12
C+X except 9	15	33	13	40
9	23	5	8	30
D	7	7	4	7
16	10	4	8	4
17-18	5	4	4	3
F	6	4	4	3
G	3	2	2	2
Y	1	2	2	2
Total	102	107	71	142

Table III. Exchanges between homologous and non-homologous chromosomes

	Exchanges between homologous chromosomes				Exchanges between non-homologous chromosomes				Total
	1×1	9×9	16×16	others	1×9	1×16	9×16	others	
Normal	9	14	4	6	7	4	2	56	102
	1×1	9×inv(9)	16×16	others	1×9	1×16	9×16	others	
inv (9)	8	3	3	9	4	4	1	39	71

as isochromatid-breaks. The heterochromatic regions of the chromosomes were particularly affected in the most cases. The number of induced exchanges and breaks observed is higher after treatment with MMC at 5×10^{-7} g/ml than at 1×10^{-7} g/ml (Table I). The number and distribution of exchanges and breaks in the individual chromosomes or groups are shown in Table II. It was noted then

Fig. 1. Examples of chromatid exchanges and breaks in chromosome No. 9 induced by mitomycin C, 5×10^{-7} g/ml. a: Homologous chromatid exchange in normal No. 9 chromosomes. b: Homologous chromatid exchange in normal No. 9 and inverted No. 9 chromosomes. c: Isochromatid break in normal No. 9 (upper) and chromatid break in inverted No. 9 (lower). Note the break points identical with those of exchanges in Fig. 1b.

that the majority of exchanges occurred at the regions of the secondary constrictions of chromosomes Nos. 1, 9 and 16, or in the so-called C-bands, whereas the distribution of chromosome breaks differed markedly from that of the exchanges. There was an overrepresentation of homologous chromosomes in exchanges involving the three chromosomes (Table III). Many homologous chromatid exchanges were also observable in the centrometric heterochromatin segments of chromosomes Nos. 1, 9 and 16 (Fig. 1a). This agrees with the findings of several other reports (Nowell 1964; Cohen and Shaw; Brøgger and Johansen 1972). However, the frequency of the exchanges was significantly reduced in normal No. 9 and inverted No. 9 (Table III, Fig. 1b), while in contrast, the frequency of breaks increased remarkably in the inverted chromosome No. 9. The break points of breaks in the inverted No. 9 chromosome correspond well to those in exchanges between the normal No. 9 and inverted No. 9 in the same cell from the treated sample. Fig. 1c exhibits breaks in both normal No. 9 chromosome (upper) and inverted chromosome No. 9 (lower) in the same cell, in which break points were identical between the two chromosomes. This seems to suggest that the pair-

ing in the inverted material is rather incomplete because of the absence of homology. The present results agree with the findings reported by De Marco *et al.* (1975) in *Drosophila* and by Heemert (1977) in onion fly. Information is now very limited concerning somatic pairing of chromosomes with rearrangements, such as pericentric inversions, in mammalian and human cells. Thus, further investigations are requested, in view of the fact that a very similar feature has been provided in both insect and human materials.

Acknowledgements. The author expresses his sincere thanks to Emeritus Prof. Dr. Sajiyo Makino, Hokkaido University, for his kind suggestions and reading the manuscript.

This work was supported in part by grants from the Ministry of Education of Japan (No. 854138).

References

- Nowell, P. C. (1964): *Exp. Cell Res.*, **33**, 445-449.
Cohen, M. M., and Shaw, M. W. (1964): *J. Cell Biol.*, **23**, 386-395.
Brøgger, A., and Johansen, J. (1972): *Chromosoma (Berl.)*, **38**, 95-104.
Rao, R. N., and Natarajan, A. T. (1967): *Genetics*, **57**, 821-825.
Kobayashi, J. (1960): *Cytologia*, **25**, 280-288.
Hansmann, I. (1976): *Human Genet.*, **31**, 247-262.
Shiraishi, Y., and Makino, S. (1977): *Proc. Japan Acad.*, **53**, 79-82.
Marco, A. de, Cozzi, R., Toti, L. (1975): *Genetica (Den Haag)*, **45**, 489-496.
Heemert, C. van (1977): *Chromosoma (Berl.)*, **59**, 193-206.